Remarks

The Invention

The invention features a method for recombinase mediated expression cassette exchange (RMCE) for substituting a positive-negative selectable marker by an incoming DNA, using FLP-recombinase.

The Office Action

Claims 1 -4, 6, 10 and 11 are pending in this application. Claims 1 -4 and 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Schlake and Bode (Biochemistry 33: 12746-12751, 1994).

Further, claims 1, 10 and 11 are rejected under 35 U.S.C. § 103(a) as the being unpatentable over Schlake and Bode in view of Jung et al. (Science 259:984-987, 1993) as well as Schlake and Bode in view of Ludwig et al. (Transgenic Research 5:385-395, 1996).

Further, claim 10 is rejected under 35 U.S.C. § 112, 1st paragraph, because the specification does not reasonably provide enablement for the practice in animals other than mice.

Rejection under 35 U.S.C. § 112, 1st paragraph

Claim 10 is rejected under 35 U.S.C. § 112, 1st paragraph. The Examiner indicated that claim 10, although enabled for use of a mouse ES cell, was not enabled for use of cells from all vertebrates. Therefore, claim 10 has been amended. The amended claim is believed to be in allowance.

Rejection under 35 U.S.C. § 102(b)

Claims 1-4 and 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Schlake and Bode (Biochemistry 33: 12746 - 12751, 1994). Specifically, the Office points to the teaching of Schlake and Bode and states that this document teaches a method of RMCE as set forth in the instant specification, in particular, the Office points out that Schlake and Bode use CV- 1 and BHK cells, but Schlake and Bode teach that one can use the technique in other cell types including mouse ES cell lines. Applicants respectfully traverse this objection. In support of their argument, Applicants submit the declaration of Alfred Nordheim, an expert in the field of stem cell research, particularly regarding embryonic stem cells.

First of all, the relevant passage of Schlake and Bode, page 12746, left column, 1st paragraph, read as follows:

> For higher eukaryotes, homologous recombination is an essential event participating in processes like DNA repair and chromatid exchange during mitosis and meiosis. Recombination depends on two highly homologous, extended sequences and several auxiliary proteins, only part of which has been identified. Strand exchange can occur at any point between the regions of homology, although particular sequences may influence efficiency. These processes can be exploited for a targeted integration of transgenes into the genome of certain cell types like embryonic stem cells. On the other hand, cultured cell lines relevant for genetic engineering purposes have lost the potential to perform homologous recombination at the efficiency that would be required to incorporate it into routine procedures (S. Karreman, GBF, unpublished). We chose BHK, which is one of the two most frequently used lines in biotechnology and has a long track record for the safe production of vaccines.

Examiner states that there is no specific teaching away from using embryonic stem cells in Schlake. Applicants, as well as Professor Nordheim, an expert in the field, disagree. From the full citation of the relevant paragraph, it is clear for a person skilled in the art that Schlake and Bode did explicitly decide to not use embryonic stem cells due to their know drawbacks. They wanted to use an established cell line, namely BHK and CV-1 cell

lines because cell lines have lost the potential for performing homologous recombination. Professor Nordheim states on page 3 of his declaration:

> Schlake and Bode were aware of the problems connected with homologous recombination occurring in embryonic stem cells and therefore chose to use established cultured mammalian cells (BHK or CV-1 cells) because they have lost the potential to perform homologous recombination. According to the understanding of Schlake and Bode especially BHK cells have a long track record for the safe production of vaccines.

Throughout the entire reference, Schlake and Bode do not use, discuss or mention the potential use of embryonic stem cells (see in particular the section "discussion" on pages 12750-12751). Therefore, Schlake and Bode do not teach the feature that the method for recombinase mediated expression cassette exchange ("RMCE") has to be performed in embryonic stem cells, as is claimed by independent claim 1.

Nor do Schlake and Bode teach the features mentioned in step (d) of claim 1, namely "maintaining the conditions for positive selection during cultivation of said cells obtained in step b) **until exchanging said first DNA expression cassette against said incoming second DNA expression cassette**." The relevant passages in Schlake and Bode are on page 12746, right column, 2nd paragraph, where it is said that "the vector is suited for positive (hygromycin) and negative (gancyclovir) selection" and page 12747, right column, 3rd paragraph, where it is stated that "BHK cells containing a single copy of F_5HygTKF (...) were cultured continuously for 4 weeks (...) **before** they were transfected with 1 μg of F_5NeoF and 2 μg of p0G44. G418-resistant clones isolated after two more weeks were characterized using PCR primers...."

From these passages, it is clear for the person skilled in the art that Schlake and Bode **does not teach** to maintain positive selection conditions with hygromycin **until exchanging** of the first DNA expression cassette against the second incoming DNA expression

cassette is complete. Professor Nordheim agrees with Applicants, stating on page 3 of his declaration:

> Schlake and Bode used hygromycin B and gangliclovir for selection (page 12747, left column, fourth paragraph), however, selection conditions are not described as set to maintain the positive selection by hygromycin all the time until the exchange of the first DNA expression cassette against the second incoming DNA expression cassette is completed.

For anticipation under 35 U.S.C. § 102, the reference must teach **every aspect of the claimed invention** either explicitly or impliedly. Any feature not directly taught must be inherently present (see MPEP § 706.02). As Applicants have convincingly demonstrated that the reference Schlake and Bode does not teach every aspect of the claimed invention, the Examiner is respectfully requested to withdraw the anticipation rejection.

Rejection under 35 U.S.C. § 103

Claims 1, 10 and 11 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Schlake and Bode in view of either Jung et al. or Ludwig et al. The Office points to the fact that Schlake and Bode mention mouse ES cell lines, but they do not teach to use the method to create cells which are capable of regenerating an animal. However, Jung et al. teaches a method using an ES cell modified by FLP recombinase to generate a transgenetic animal. Therefore, the Office believes that it would have been *prima facie* obvious for a person skilled in the art at the time the invention was made to use the methods of Schlake and Bode to modify the genome of an ES cell using FLP recombinase to create a transgenic animal as described by Jung et al.

Further, the Office believes that Ludwig et al. is equally suitable to be combined with the disclosure of Schlake and Bode, because Ludwig et al. teaches a method to modify the genome of a fertilized one cell egg using FLP recombinase. Therefore, it would have been *prima facie* obvious for a person skilled in the art at the time the invention was made to use the methods of Schlake and Bode to modify the genome of fertilized egg using FLP

recombinase to create a transgenic animal as described by Ludwig et al. Applicants respectfully traverse this rejection.

To make out a *prima facie* case of obviousness, the Patent Office must show that "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person skilled in the art to which said subject matter pertains." 35 U.S.C. § 103(a) (emphasis added). The framework for making such a determination was set out by the United States Supreme Court in *Graham v. Deere Co.*, 383 U.S. 1, 17-18 (1966):

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined.

Obviousness cannot be established by combining the teaching of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. *ASC Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 U.S.P.Q. 929, 933 (Fed. Cir. 1984). This suggestion or motivation may be derived from the prior art reference itself, from the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. The basis for a prior art combination, however, must come from a source other than the inventor's disclosure. The Court of Appeals for the Federal Circuit has repeatedly emphasized that hindsight analysis is an inappropriate means for piecing together the elements of an invention from unrelated references. For example, in *In re Fritch*, 972 F.2d 1260, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992), the Federal Circuit stated:

> It is impermissible to use the claimed invention as an instruction manual or 'template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious.

And in *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985), the court stated:

> It is error to reconstruct the patentee's claimed invention from the prior art by using the patentee's claim as a "blueprint". When prior art references require selective combination to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight obtained from the invention itself.

Applying these standards to the present case, it is clear that the references cited in the latest Action do not support a *prima facie* case of obviousness. Applicants also submit the declaration of an expert in the field, Professor Nordheim, in support of their traverse of this rejection.

First, none of the references teach or suggest the features mentioned in step (d) of claim 1, namely "maintaining the conditions for positive selection during cultivation of said cells obtained in step b) **until exchanging said first DNA expression cassette against said incoming second DNA expression cassette**." From passages cited above, it is clear for the person skilled in the art that Schlake and Bode **does not teach** to maintain positive selection conditions with hygromycin **until exchanging** of the first DNA expression cassette against the second incoming DNA expression cassette is complete. Nor do Jung et al. and Ludwig, et. al. teach this claim limitation. Thus, even if there were a suggestion in the art to combine these references, the combination would not render the claims obvious because all the limitations are not present.

In addition, Schlake and Bode, as already discussed above, clearly state to not use embryonic stem cells but rather suggests to use established cell lines like BHK or CV-l cells due to their known advantages. Therefore, a person skilled in the art would never have started with the document of Schlake and Bode and combine it with either Jung et al. or Ludwig et al.

Moreover, as Professor Nordheim states on page 7 of his declaration:

> Combining the teachings of Schlake and Bode with either Ludwig et al or Jung et al, the person skilled in the art would

> not teach the claimed invention. The prior art references ... use embryonic stem cells and murine embryos respectively, the technology disclosed is either classical site-specific recombination and/or homologous recombination, which all comprise major drawbacks discussed in the invention. **Therefore, a person starting from the disclosure of Schlake and Bode would not gain any further information from Jung et. al. or Ludwig et al. in order to reach the claimed invention. In particular, a person skilled in the art would not be able to solve the problem of integrated vector sequences or low efficiencies in recombination together with the requirement for an incoming selectable marker.**

Finally, secondary considerations warrant against a finding of obviousness. Schlake and Bode observed targeting frequencies for F3 of .24, .51 and 1.5 % in the BHK and VC-1 cell lines they felt were best suited . In contrast, the claimed invention exhibited targeting frequencies of F18, F21 and F22 of 100%, 54& and 100%, respectively (see page 5 of Nordheim declaration.) Thus, the prior art did not exhibit or ever expect to achieve the high targeting frequencies of the present invention. Indeed, much lower frequencies than that obtained in Bode and Schlake would have been expected in embryonic stem cells.

A person skilled in the art may thus not have been able to reach the claimed invention by combining the teaching of Schlake and Bode with either Jung et al. or Ludwig et al.

The § 103(a) rejection in this case should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such Action is respectfully requested.

A check in the amount of $~~165~~ 210.00 is enclosed to cover the Petition fee. Please charge any additional fees or credit any overpayments as a result of the filing of this paper to our Deposit Account No. 02-3978 -- a duplicate of this paper is enclosed for that purpose.

Respectfully submitted,

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